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Throughout the last decade, the sucking insect populations have increased due to the widespread adaptations of transgenic Bt cotton and altered chemical control methods. *Lygus lineolaris* is one that is among this increasing type of bug populations and is considered to be the most economically significant. *Lygus lineolaris* is more commonly known as the Tarnish Plant Bug or TPB for short. Chemical control is what the management of the TPB relies on, almost exclusively. However, in recent years, the TPB has become increasingly resistant to organophophates. Other commonly other than organophophates used insecticides includes pyrethroids, carbamates, and neonicotinoids. The most widely used insecticide for TPB control

would be an organophosphorus insecticide that goes by the name of Acephate or more commonly known as Orthene.

Cotton is more frequently sprayed compared to the other major crops in the south in order to ord

suppress damages due to feeding of the TPB. Several insecticides, including Acephate, TPB have become increasingly resistant towards and some of these areas that are found in the Mississippi, Arkansas, and Louisiana area show a 3- to 5-fold increase. Two strains which were a susceptible (LLS) and an acephate-selected (LLR) strains were systematically conducted by biological, biochemical, and molecular experiments in order to better understand acephate resistance mechanisms.

In order to see how susceptible or resistant the TPB is to insecticide, different types of experiments are performed including bioassays and enzyme assays. In order to perform these

tests, there must be a control group. There was a control group made from the colony that was provided by Kathy Knighten and Fred Musser at Mississippi State University. The LC_{50} was calculated for the field and laboratory strains of the LLS. To provide the LLR, bugs were collected off of pigweed around fields in Lula, MS. Of the Lula collected bugs, acephate-selected bugs, named Lula600, was created by performing an acephate treatment on them. After collecting these 45,000 bugs, they were treated with Orthene 90WP at 600 mg/L. After treatment for 12 hours, the bugs were then moved into a different cage with fresh green beans.

After 6 days of the first acephate treatment, a dose-response bioassay was performed on the survivors. This involved having six acephate concentrations at 300, 500, 750, 1,000, 1,500, and 2,000 mg/L in d-H20 as well as a water control. There were three replicates for each concentration and 17 bugs were placed into each replicate. The adults with four fresh green beans were transferred into a clean plastic container after acephate exposure for 10 minutes. After 48 hours, mortality was then recorded and the LC_{50} was calculated using the SAS Probit analysis.

The other test, called the enzyme activity assay, tests for esterase and glutathione S-transferase enzyme activity. Using protocols described by Zhu et al., nine Tarnish Plant Bugs from each of the samples were homogenized in sodium phosphate buffer. For 5 minutes at 4°C, the homogenate was centrifuged at 10,000 x g. In order to determine the protein concentrations, the Bradford method was utilized along with using the Pierce protein assay kit. There were two machines that collected enzyme assay data including the Bio-Tex Powerwave HT plate reader and the ELx808iu plate reader. To test for esterase activity, a-NA, (3-NA, and

PNPA were used as substrates in the micro-titer plate assays. Also, to test for glutathione S-transferase activity, CDNB was used as a substrate in micro-titer plate assays. Each of the micro-titer plate assays were put into the specific machine and were monitored at some level of nm for 10 minutes with every 15 seconds readings.

The results of bioassay showed an increase of the LC_{50}forthe LLR strain compared to the LLS strain—by a 2.2-fold increase. After treatment of the Lula600, the LC_{50} reached a high level of 5.9-fold higher than the LLS. The results of the enzyme assay with a-NA showed that there was an increase in esterase activity in the acephate selected bugs (Lula600) by a 4.5-fold increase and a 2.5-fold increase in Lula field population than the LLS. There was a 2.8-2.9- fold increase with Lula600 using PNPA and a 1.3-1.4 fold increase in Lula with J3-NA. However, no increase in glutathione S-transferase was recorded and actually there was a decrease in levels of this enzyme found in the acephate-selected bugs.

These experiments proved that the more exposed the bug is to the insecticide, the more resistant the bug will be. In turn, the more resistant the bug is, the more up-regulation of genes for the specific enzymes will occur. This is explained because the more genes that the bug has to code for the enzymes that break down the chemicals that are found insecticide, the better the chance it has to survive. This research study was a way to allow for the understanding of the molecular composition with regards to the resistance of the Tarnish Plant Bug while proving information that will be of benefit in development of chemical controls to minimize resistant with this same bug in the future.

Works Cited

Zhu YC, Guo Z, He Y, Luttrell, R, (2012) Microarray Analysis of Gene Regulations and Potential Association with Acephate-Resistance and Fitness Cost in *Lygus Hneolaris.* PLoS ONE 7(5): e37586. Doi:10.1371/journal.pone.0037586